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Abstract: Contamination of agricultural soils with trace elements (TEs) through municipal and industrial wastes, atmospheric deposition and fertilisers is a matter of great global concern. Since TE accumulation in edible plant parts depends on soil characteristics, plant genotype and agricultural practices, those soil- and plant-specific options that restrict the entry of harmful TEs into the food chain to protect human and animal health are reviewed. Soil options such as in situ stabilisation of TEs in soils, changes in physicochemical parameters, fertiliser management, element interactions and agronomic practices reduce TE uptake by food crops. Furthermore, phytoremediation and solubilisation as alternative techniques to reduce TE concentrations in soils are also discussed. Among plant options, selection of species and cultivars, metabolic processes and microbial transformations in the rhizosphere can potentially affect TE uptake and distribution in plants. For this purpose, genetic variations are exploited to select cultivars with low uptake potential, especially low-cadmium accumulator wheat and rice cultivars. The microbial reduction of elements and transformations in the rhizosphere are other key players in the cycling of TEs that may offer the basis for a wide range of innovative biotechnological processes. It is thus concluded that appropriate combination of soil- and plant-specific options can minimise TE transfer to the food chain.

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Safety of food crops on land contaminated with trace elements

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Abstract

Contamination of agricultural soils with trace elements (TE) through municipal and industrial wastes, atmospheric deposition and fertilizers is a matter of great global concern. while the demand for safe food is ever increasing. Trace elements accumulation into edible plant parts depends on soil characteristics, plant genotypes, and agricultural practices. Therefore soil and plant specific options that restrict the entry of harmful trace elements into food chain and thus protect human and animal health are reviewed. Soil options such as in situ stabilization of TE in soils and changes in physico-chemical parameters, fertilizer management and element interactions, and agronomic practices are reducing TE uptake by food crops. Decontamination techniques such as phytoremediation and solubilization are alternatives to reduce TE concentrations in soils. Among the plant options, selection of species and cultivars, metabolic processes, and microbial transformations in the rhizosphere potentially can affect TE uptake and distribution in plants. Genetic variations across crop species and cultivars are exploited to

select low uptake potential cultivars, especially low-Cd accumulator wheat and rice cultivars. Metal-ligand speciation and metal transport in plants are dynamic, changing across tissues, sub-cellular compartments, developmental stages, and plant species. The microbial reduction of elements and the transformations in the rhizosphere are key players in the cycling of both inorganic and organic substances and thus they may offer the basis for a wide range of innovative biotechnological processes. The combination of soil and plant specific options can optimize the TE transfer to the food chain.

Key words: Cultivars, food safety, immobilization, land contamination, metal transport, microbial transformation, molecular process, plant species, phytoremediation, rhizosphere, trace element.

1 Introduction

The production of quality food depends on the availability of fertile, uncontaminated soil, an adequate supply of moisture and nutrients and on the biological functioning of the agro-ecosystem. In addition to inputs of trace elements (metals and metalloids) through fertilizers, pesticides, and atmospheric deposition, inadvertent use of municipal and industrial wastes, or recycling of animal manure, especially pig slurries, containing essential nutrients but also significant levels of potentially toxic TE, are major sources increasing the contamination of agricultural soils and thus a matter of growing global concern.

Some TEs, e.g. copper (Cu) molybdenum (Mo) and zinc (Zn), are essential for plant growth and human and animal nutrition, but can create phytotoxicity and/or zootoxicity concern when accumulated in excessive concentration in the soil and plants. Other TEs such as cadmium (Cd), arsenic (As), mercury (Hg), and lead (Pb), not essential for either plants or human and animals, pose risks when entered to food chain through contaminated soil amendments, fertilizers, and other anthropogenic sources. Although contaminants in food and fodder do not induce quick death, they cause chronic health effects. Therefore the contaminant's ability to release TEs should be properly regulated through agronomic, biochemical, and physical factors and decontamination programs.

The potential TE uptake by roots, from either anthropogenic or geochemical sources, depends on their total concentration in the soil, soil solution and exchangeable forms. Levels of contamination by TE in soils, the actual risk, agronomical regions at major risk, the TE

levels in soil that would trigger soil management measures, the crops more sensitive (e.g. for Cd accumulation, leafy vegetables, carrots, rice, and durum wheat) to TE pollution and elements of major concern, such as Cd, As, Hg and Pb, are detailed elsewhere[1,2,3,4,5,6,7,8,9,10,11,12,13,14,15,16,17,18]. Metal inputs differ among the EU countries though within the upper maximum limits as stipulated in the EU Directive. Likewise, the loading in the US, Canada and Australasia are different. Hooda (2010)[18] provides an overview of the different regulatory limits.

The chemical behaviour of TEs varies from soil to soil and is influenced by soil properties, such as pH, organic matter content, clay and amorphous hydrous oxide contents and cation exchange capacity[1, 19]. However, the TE accumulation and transport of in plants to harvested and edible parts also depends on biotic factors. Those affecting the uptake of TE are crop species and cultivars, root activity, rooting patterns and rhizosphere associated microorganisms[20, 21]. For example, crop Cd is not simply function of total soil Cd but depends on, e.g. soil Cd binding strength, soil pH and soil chloride, plant species and cultivars, and preceding crop [1,10,17,22]. Few crops are at risk from usual Cd contamination sources (Zn mine wastes and smelter emissions), except for rice soils[23]. High Cd phosphate fertilizers, high Cd biosolids, and mineralized soils affected by marine phosphorites cause Cd contamination with little Zn contamination so the Cd is much more phytoavailable and bioavailable. Cases of Cd contaminated agricultural products occurred in some areas, e.g. Kempen, an area that comprises eastern Flanders, Belgium and the adjoining part of the Netherlands, and rural areas around Evin-Malmaison, Auby, and Mortagne-du-Nord in Northern France due to historical smelter emissions [2, 24] Based on soil survey from 11 provinces in China, at least 13,330 ha farmland has been contaminated by Cd and reduction of crop Cd is an increasing health issue for China[25]. Risk from Cd should consider the nature of the Cd source, presence of normal Zn levels, and Cd bioavailability in the crop [23]. Present international limits on crop Cd which assume that Cd has equal bioavailability or risk in all foods, and that Zn in the food has no affect on Cd bioavailability are controversial[23] . People who are nutritionally marginal with respect to Zn, Fe, and Ca are at higher risk of Cd disease than those who are nutritionally adequate. Urinary level of 1–2 μg Cd/L is associated with an increased risk of bone demineralization and fractures, and 2–4μg Cd/L with pre-clinical kidney damage [26] . People with excessive, chronic Cd exposure may develop the adverse health effects at an old age,

when Cd has accumulated in the body.

Agronomic practices which impact on TE concentration in the soil solution by affecting the above mentioned chemical properties or on crop growth and rooting patterns can affect the accumulation of TEs in plants. Therefore, the strategies to restrict TE entry into food chain must include both soil specific options (agronomic management) and plant specific options (species and cultivars, understanding at molecular level, and plant roots and microbial transformation in the rhizosphere).

Contaminated soils pose a serious threat to healthy food production and hence their remediation is required. Remediation of contaminated soils using conventional clean- up technologies is expensive and not feasible for large agricultural areas. Where TE can be tightly bound to the soil constituents and are not bioavailable as evidenced by bioavailability bioassays and other toxicity assays, exhaustive clean-up of soils may not be necessary as the contaminants may not pose a risk to end users. This management option is termed '"risk-based land management'" (RBLM) and is considered attractive as it may save millions of Euros in remediation costs. Despite the importance of bioavailability, there is still considerable controversy regarding its definition and also what constitutes the bioavailable fraction in soils.

Uptake and distribution of TE, especially of Cd, differ widely among plant species and cultivars [20,25,27,28,29] This could partly be related to differences in the abilities of plants to control movement of TE from the xylem into the phloem and via the phloem into the seeds [30,31]. Therefore plant breeding and selection can be an important tool to reduce the potentially harmful TE such as Cd in food crops[22]. Similarly, TE uptake in edible plant parts can be restricted from soil to root, reduction in influx across root, accumulation in vacuoles and flux back to non edible plant parts.

Plants and microorganisms exude a variety of inorganic and organic substances that may alter soil pH and directly influence TE availability through solubilization and complexation. Factors influencing TE fractionation and bioavailability in soil include root-induced pH changes, TE binding by root exudates [32,] root-induced microbial activities[21], and root depletion as a consequence of plant uptake. The processes affecting rhizospheric pH involve the evolution of CO_2, the release of root exudates, the excretion or re-absorption of H^+

or HCO_3-, and the microbial production of organic acids [33]. The microbial reduction of elements is gaining interest because these transformations can play crucial roles in the cycling of both inorganic and organic compounds in a range of environments. If harnessed, it may offer the basis for a wide range of innovative biotechnological processes.

In spite of our increased knowledge on TE in soil-plant-human system, the complex web of geochemical and biological interactions limit the prediction of the TE bioavailability for plant uptake and their assimilation in human and animals. This review focuses on soil and plant specific options restricting the entry of harmful trace elements into food chain and thus protecting human and animal health. Among the soil specific options, emphasis is placed on in situ TE stabilization in soils by sorbing agents (e.g. mineral oxides, manure and organic materials, phosphates, clays, etc.), changes in physico-chemical parameters (e.g. addition of alkaline materials, coal fly ashes, etc), fertilizer management and element interactions and agronomic practices (crop rotation and tillage)[1,34,35,36]. For plant specific options, selection of species and cultivars, metabolic processes (e.g. metal binding by proteins, detoxification by glutathione or amino acids), and microbial transformations in rhizosphere affecting TE uptake and distribution in plants are discussed. Although TE such as Cd, Hg, Pb, As and Se are of major concern with respect to human food-chain, Cd gets major focuses here because its labile pool is relatively important in soils facilitating plant uptakes and Cd is extremely hazardous to animal and human health.

2. Site specific management of contaminated soils/sites

The risk reduction in contaminated sites are principally can be carried out by various soil management techniques. These are mainly divided in two categories: stabilization or decontamination (Table. 1). The choice of the principal category is mainly made on different site factors such as soil type, the nature and distribution of pollution as well as the severity of the hazard, current land use, soil pH, and clean-up goals[37]. With knowledge of these major points, the decision can be taken whether stabilization or a decontamination procedure is preferable. Knowledge of the current land use will reveal whether or not changes are needed. If the pollutants should be stabilized, the pH of the soil makes it clear whether liming or another stabilization technique should be applied. When the final goal is a complete decontamination of the soil, further investigations are necessary to determine the appropriate decontamination technique. Up to now, most *in situ* remediation techniques are still at an

experimental stage and are not adapted to a large spectrum of soil types or various pollutants[38].

In the following sections known and new possible techniques are critically evaluated and presented in detail. Our concept of gentle remediation is not restricted to either stabilization or decontamination. In a remediation process stabilization may only be the first step which reduces the hazard and gives time to make detailed investigations to optimize the following stabilization and decontamination[38].

2.1. Soil approaches (agronomic management

2.1.1. In situ stabilization through different binding agents

Contrary to organic pollutants, TEs are not subjected to decomposition processes and hence they may cause persistent contamination. To manage TE contaminated soils various remediation techniques are proposed (Table1). Most techniques aim at protecting humans, animals and the environment from exposure to hazards by removing the source or interrupting the pollutant linkages. Two major categories of remediation and clean-up techniques can be distinguished: Techniques that enable the long-term restoration and preservation of soil fertility, so-called "gentle" remediation techniques, and harsh clean-up techniques that primarily aim at eliminating human health risks. Most harsh techniques impair biological activity or destroy the physical structure of soil.

Gentle remediation techniques are designed to eliminate hazards without destroying the soil, i.e. without affecting biological activity, soil organisms, chemical properties and physical structure. They are in particular required where large areas of low TE contaminated agricultural land need to be remediated. The main principle behind in situ stabilization is to render TE in unavailable or inactive form [35, 39, 40]. In situ stabilization can reduce the mobile and bioavailable TE fractions in soil (Fig. 1), avoid their migration into the ground water, limit the uptake of trace elements by plants and thus reduce their toxicity to plants[41, 42, 43]. In situ stabilization increases the sorption capacity of the soil matrix by addition of agents such as clay minerals, Fe-, Mn-, Ti- and Al-oxides, phosphates, OM, etc. or decreases the concentrations of dissolved pollutants by changing soil parameters such as pH (e.g. dolomitic limestone, coal fly ashes, etc) and redox potential (Eh).

2.1.1.1. Liming

Liming can reduce the mobility of TE, such as Cd, Ni and Zn [44, 42, 35]. Its effect on Cu solubility and plant uptake is more complicated due to the formation of complexes with soluble organic substances after liming [45]. Although liming has proved to be efficient for minimizing risks that TE pollutants pose by entering into the food chain, its effects can vary considerably depending on TE, soil conditions, plant growth and especially root distribution in the soil. Also liming provides only a transitory solution to the pollution problem and in light textured soils, overliming may also decrease availability of essential micronutrients such as Zn and Fe. ,

The "soluble" TE fraction in soils can be reduced by rising pH (e.g. liming, alkaline fertilization) or by increasing cation binding capacity (e.g. addition of clay minerals or gravel sludge)46,47. Liming decreases NH_4 NO_3-extractable fraction of TE (e.g. Cd) in soils and reduces the uptake of Cd, Ni and Zn by wheat and carrot crops grown in naturally metal- rich soils (alum shale soils) in Norway[47] (Table 2). Excessive application of sewage sludge and pig manure lead to the accumulation of potentially toxic elements in soil but liming reduced the solubility and plant uptake of Cd and Zn[27]. However, the reduction was higher in control plots than in sludge treated plots showing the interaction between TE with soil pH, organic matter (OM), root distribution and rhizospheric interactions. In contrast to general decrease in TE concentration after liming, some studies reported increased Cd concentration in crops [48, 49]. The Ca^{2+} added through lime may desorb surface bound Cd^{2+} into soil solution, rendering it available to plants. Calcium is inhibiting Cd^{2+} sorption to soil surface, but the mechanism can only have a significant effect if the pH induced increase in sorption of Cd^{2+} with liming is less than the Ca-induced desorption of Cd[19,50] .

2.1.1.2 Trace element binding materials

Binding agents that increase sorption capacity in soil matrix include the application of chelate, ion exchange resins or natural materials such as organic substances or clay minerals[51]. Out of 20 different additives tested in batch and column experiments, zeolite combined with ferrous sulfate was effective in immobilizing Cd in different soils[52]. Alkaline fly ashes reduce the metal availability and decrease TE uptake by maize[53]. Another coal fly ash called beringite, which is a modified alumino-silicate from the fluidized- bed burning of coal mining in Belgium has been tested[54, 55]. In field experiments, the beringite addition to a soil polluted with 6000 mg Zn, 30 mg Cd and 500 mg Cu kg^{-1} enabled the re-establishment of plant growth and protected the polluted area from erosion. The 5% addition of beringite to a Zn-

contaminated soil reduced foliar Zn concentration in bean (*Phaseolus vulgaris*.) from 350 mg kg^{-1} in the untreated soils to 146 mg kg^{-1} in the beringite-treated soil[56]. Similar beneficial effect of beringite (2.5 and 5 % addition) are obtained with plant growth and Cu uptake by bean (*Phaseolus vulgaris L*) and maize (*Zea mays* L.) grown in a Cu-contaminated soil (250 mg kg^{-1}) from coffee orchards in Tanzania [57.] Increased pH and Cu sorption by the soil are the suggested mechanisms.

The potential of montmorillonite (MMT), Al- MMT and gravel sludge for the immobilization of TE in agricultural soils was investigated[58]. In batch experiments, both Al-MMT and MMT are effective in immobilizing Zn and Cd. Zinc is specifically bound on Al-MMT and in the time course it becomes increasingly incorporated into the Al hydroxide coatings. No specific Zn sorption occurs on montmorillonite. Cadmium is bound on MMT and Al-MMT non-specifically by cation exchange processes

In a pot experiment, zeolites reduce TE uptake, but the reduction was partially caused by a pH increase as side effect of zeolite[59]. Regarding TE leaching, TE concentration in effluent was 50% lower in zeolites-treated column than in the $CaCO_3$–treated soils. Some adverse or subsequent effects such as the immobilization of nutrients (P, Mn) may occur[60.] Effect on foliar Ca, Mg, K and P concentrations of bean is reported in stabilized Cu-contaminated soil[45.] The remobilization by soil acidification have not yet been investigated and similar to remediation with liming, the long-term behavior of immobilized metals in different soils is generally unknown and the database of long-term field studies is weak [61] .

2.1.1.3 Trace element reaction with iron and manganese oxides

Sorption is an important chemical process that regulates TE partitioning between solution and solid phases in soils. Iron and manganese oxide minerals are important sinks for TEs in soil [62, 63] and residual-amended soils[64, 65]. Hydrous ferric oxide decreases the extractable Cd in the soil but reduced uptake by plants could not be measured[66]. Root exudates may dissolve the hydrous ferric oxide and as a result the element became available. Other iron bearing products, e.g. Fe-rich adsorbent[67, 68,] zerovalent iron grit [35, 36, 40] have been studied. The combination of zerovalent iron grit and beringite is very efficient to stabilize metals and As[60, 55, 69, 40]. However long term evaluation for an As/Zn contaminated spoil showed As concentrations in leachates percolated from the remediated spoils were higher than those of the unamended spoil[70] Manganese oxides are important adsorbent and one most reactive form is synthetic birnessite (sometimes called δ-MnO_2)[71] . In a study on the immobilizing capacity

of birnessite, it was found that birnessite exhibited the best potential in reducing the uptake of Cd and Pb by plants as compared with other additives (hydrous ferric oxide, basic slag, beringite, and lime)[51]. However, under reducing conditions the Mn oxides may reduce to Mn^{2+}, which can be toxic for organisms.

2.1.2. Solubilization of trace element by ligands to enhance plant uptake

To enhance TE uptake by the use of chelators the following steps are necessary: TE must be (1) dissolved from the solid, (2) transported to the plant roots, (3) absorbed by the roots and (4) translocated within the plants to the above ground parts. The solubilization process must be carried out with caution in order to avoid loss of TE by leaching to the groundwater but at the same time provide an optimum concentration of soluble TE in the root zone, available for removal by plants [72, 73]. This concentration must maximize plant uptake, but does not induce growth reduction. For maintaining such optimum concentration during the vegetative period, it might be necessary to add the amendment several times at a low dose... The optimum time span between two treatments depends on the degradation rate of the applied ligand. In order to minimize leaching, the chelator application should be restricted to the root zone.

The formation of soluble metal complexes may not necessarily lead to enhanced metal phytoavailability. While some authors report that the use of chelators such as EDTA (ethylenediaminetetraacetate) increased metal uptake by plants[74], others did not observe an enhancement[75] but rather a reduction of metal uptake by plants. Furthermore, the addition of chelators may have undesired side-effects such as increasing the metal toxicity, as well as the risk of metal leaching to deeper soil layers or to groundwater.

The influence of natural organic agents, citric, oxalic, phtalic and salicylic acid and three synthetic organic agents, EDTA, NTA (nitrilotriacetate), and DTPA (diethylenetriaminepentaacetate) on metal solubilization in soils was studied in batch experiments[72]. Experiments were performed with soils from two metal-contaminated agricultural sites of northern Switzerland contaminated with Zn, Cu, and Cd, while one was also polluted by Pb. The chelator efficiency was far better than that of the natural organic agents. Despite substantial differences in stability constants, there were not much significant differences among NTA, EDTA and DTPA in extracting metals from the two soils. Considering the high degree of biodegradability of NTA in soils, it was chosen as substance

to be used in phytoextraction experiments.

Pot and field experiments were conducted to investigate the effectiveness of NTA (chelator) and elemental sulphur (agent to lower soil pH) on metal solubilization and uptake by *N. tabacum* and *Z. mays* [73]. Potential harmful side effects such as metal leaching to deeper soil layers were also studied. The addition of elemental sulphur (100 mmol S kg^{-1}) increased dissolved Zn and Cd concentrations about 10 fold in the calcareous soil and up to 30 fold in the acidic soil by decreasing soil pH about 1 to 1.5 units. No effect on soil pH was observed in the NTA treatments. NTA application (0.5 mmol NTA kg^{-1} in the calcareous soil; 0.25 mmol NTA kg^{-1} in the acidic soil) increased soluble Zn, Cd and Cu about 100, 19 and 20 fold in the calcareous soil and 13, 2 and 4 fold in the acidic soil. Dissolved Pb was increased by NTA up to 50 fold in the acidic soil. Solubilizing effects remained only for 7 days and then decreased rapidly within 20 days to almost initial values.

In general, the metal solubilization treatments increase $NaNO_3$-extractable Zn, Cd, and Cu and Pb concentrations in soils[73]. However, this increase did not translate into an equivalent one in metal uptake by plants, although in nutrient solution experiments a much higher increase in Cu uptake and translocation into shoots could be observed. The lower efficiency in the soil is attributed to the short duration of solubilizing effects. The leaching of TE in these experiments was not investigated.

2. 1.2.1 Phytoremediation

Decontamination techniques include the use of hyperaccumulator or high biomass crops that accumulate high TE amounts in shoots and thus can remove TE from contaminated soils[76]. Plants represent a more environmentally compatible and less expensive method of site restoration, through extraction, degradation or fixation of the pollutants, compared to physico-chemical and engineering options, even though the time scale required reaching the fixed end-points is a limiting factor.

To overcome the limitations of phytoextraction techniques several options are taken. Efforts are made to increase growth of hyperaccumulators by crossbreeding them with related plants that produce more biomass[77] or using molecular mechanisms and genes leading to hyperaccumulation in tolerant species[78]. Additionally, attempts are made to improve metal uptake capabilities of high biomass plants by somaclonal variation or chemical mutagenesis and selection techniques[79]. Furthermore, some plants such as Salix can decrease the metal concentration in soils. For example, in a field study in Sweden, growing Salix prior to wheat crop decreased the Cd concentration significantly in soils as well as in wheat grain[80].

A different option is to increase the metal phytoavailability in soil. Soil factors primarily controlling metal phytoavailability in soils are pH, cation exchange capacity (CEC) and organic matter content[81]. For increasing phytoavailability two major ways are investigated: artificial soil acidification and solubilization by addition of ligands, in particular chelators. Citric acid and hydrochloric acid display effects, forming complexes and decreasing soil pH. For both substances, an enhancement of element uptake by plants has been reported [82, 83...]

Once mobilized in the rhizosphere, mineral elements and contaminants need to be taken up into the root. For example, the Zn hyperaccumulator *Thlaspi caerulescens* over-expresses a ZIP family root plasma membrane transporter. In the closely related non-accumulator species, T. arvense, high external concentration suppresses expression of this Zn transporter, indicating that metal regulation of gene expression is altered in the hyperaccumulator. One of hypothesis is that key genes necessary to cope with and to translocate potentially toxic TE are up-regulated in the hyperaccumulators. However, those genes also exist in sensitive species but are not expressed in the appropriate tissues or at a sufficient level.

2.1.3 Fertilizer management and trace element interactions

Fertilizers and especially phosphates contain TE impurities and can result in excessive TE in soils. Many examples of TE accumulation in soils and especially of Cd in long term fertilized soils are worldly reported[84,85,86,87] However, the increased total soil Cd is not always reflected in increased Cd concentration in food crops because fertilizers can affect TE root uptake by changing soil pH and ionic strength in the soil solution and plant growth parameters, e.g. root distribution, rhizosphere conditions, and shoot yield. The type and amount of fertilizer used and the interactions between TE and major nutrients (N, P and S) and among TE themselves (e.g. Zn - Cd and Fe - Cd) play an important role in the TE uptake in crops [88]. High Fe nutrition caused a
Marked reduction in Cd content in both leaves and roots... Iron content in plants was lower at high Cd (5.0 mM) stress than at low Cd (<1.0 mM) stress. Cadmium stress affects the uptake of Fe, Cu and Zn[89].2.1.3.1 Interaction with major nutrients

Change in TE in food crops in relation to N fertilization is difficult to evaluate because N supply affects crop yield as well as the soil reaction. Grain Cd in wheat increases with increasing N fertilization rate, except for urea[90]. The N and Cd concentrations positively correlate in wheat grain in Sweden[91, 92]. Each 10 kg N ha^{-1} additional application increase grain Cd by 1-3 µg kg^{-1} [92]. The relative increase in Cd concentration as a function of N rate

varies from 6 to14% across sites and cultivars when the N rate increases from 145 to 175 kg N ha^{-1}. The increased concentration of Ca^{2+} due to Ca nitrate application may raise ion exchange Cd into the soil solution and wheat grain Cd.

Phosphate fertilization and Cd in soils often positively correlate[93,94,95,88]. Total P and Cd are linearly related in pasture soils receiving long term input of superphosphate[95]. In Norway, long term use of P fertilizer increases total soil Cd concentration but this does not necessarily enhance the Cd uptake by plants[96]. Different P sources may affect Cd uptake differently. The Cd uptake by rape and oats in greenhouse experiment is higher with single superposphate than with NPK[97.] The single superphosphate contains 12% S and acidification due to its application could have enhanced the solubility and availability of Cd. However, the P source has only a little effect on the Cd concentration in potato tubers[98]. Potassium application may increase Cd uptake by food crop but it may be associated more to the accompanying anion of the salt. The application of KCl increases grain Cd in barley[99]. This uptake may be associated to increased soil solution concentration of Cd via formation of Cd Cl n $^{2-n}$ ion pairs[100].

2.1.3.2. Interaction with micronutrients

The Zn and Cd interaction is widely studied because these metals behave chemically similar and coexist in contamination sources. The effect of Zn fertilization would depend on the Zn status of soils and plants and significant effects are found in Zn deficient soils[101]. On a Zn deficient soil, Zn application up to 100kg ha^{-1} reduces the Cd uptake by potato tubers by 20% [88]. Also Cd competes with Cu for plant uptake[102]. Increases in seed Zn concentration, whether caused by Zn status in soil, P fertilization or application of Zn fertilizer, result in decreased Cd concentration[103]. A 20 mg Zn kg^{-1} soil application with P decreases seed / grain Cd by 42% for flax and 65% for durum wheat and Cd translocation to the seed/ grain by 20% for flax and 34% for durum wheat[104]. An antagonistic effect of Zn on Cd root uptake and translocation to seed/ grain in both crops is assumed in growth chamber studies. Increasing Cd application to Zn-deficient plants tends to decrease plant Zn concentrations, whereas in plants with adequate Zn supply, Zn concentrations are either not affected or increased by Cd[105] .Durum wheat is more sensitive to Zn deficiency and Cd toxicity than bread wheat. Authors[105] hypothesized that Zn protects plant from Cd toxicity by improving plant defense against oxidative stress and by competing with Cd for binding to critical cell constituents. Cd decreases by 11 to 90 % in wheat when Zn is applied at 15 mg kg-1 soil[106]. Because Zn

concentration grain cereals are too low to meet the nutrition requirement for humans, biofortification of cereal grain Zn would be an issue, [30]: Evidences for other interactions notably for Si are emerging. In maize seedlings, Si alleviates Cd toxicity as revealed at some antioxidant enzyme activities [107], most prominent effects being in the roots. Silicon induces Cd resistance in rice[108] and affects wheat grain Cd [109].

2.1.3.3. Salinity and chloride and sulphate ions

Chloride forms complexes with TE and especially with Cd and hence Cl supply either through fertilizers (e.g. KCl) or in irrigation water can increase Cd uptake by crops[110, 111, 82]. In Australia 70 % of variation in Cd concentration in potato tubers across 80 sites was caused by salinity and Cl supply through irrigation water[110]. Similarly, increasing salinity increases the concentration of Cd species, i.e. Cd^{2+}, $CdCl^{+}$, $CdHCO^{3+}$ and $CdCl^{0}$ in soil solution, while salinity decreases total and free Zn^{2+} concentration in soil solution and its concentration in wheat shoots [106] .The uptake of $CdSO_4$ ion pair by Swiss chard is equally efficient to uptake of the free Cd^{2+} from nutrient solution, however little or no increase in Cd uptake is observed when SO_4^{2-} is applied to soils[112].

2.1.4. Tillage systems and crop rotation

Limited information on the effect of tillage practices, i.e. conventional vs. reduced or no till, on TE concentration in food crops make it difficult to draw any definite conclusions. Wheat grain grown under direct drilling contains higher Cd compared to reduce till or conventional cultivation [90, 88]. But EDTA extractable Cd in soils is not affected by tillage practices. Tillage practice with no till system however may create the stratification of TE, i.e. Cu, Fe, Mn, and Zn, because of crop residue and OM accumulation on the surface[113]. Deep tillage could be an effective technique in conditions where surface soil is enriched with TE because it may lead to dilution of elements by blending of surface and sub-surface soils.

Rhizosphere effects of plants in crop rotation may affect the TE availability to the following crops. Lupines (Lupines L.) are known to release citric acid leading to soil acidification and consequently increased lability of elements in the soil. Grain Cd in wheat is higher after a lupine culture[90]. The increase in Cd concentration may be partially, but not solely, attributed to acidification by legumes and the subsequent Cd mobilization for uptake by the subsequent crop Cropping systems such as rotation and intercropping may have numerous advantages in terms of increasing availability of micronutrients, including Zn. In a

Chinese peanut /maize intercropping example, the phytosiderophores (PS) excretion by maize into rhizosphere plays an important role in improving Fe nutrition of peanut intercropped with maize[114]. Enhanced PS release by plants may mobilize Zn in the soil and enhance Zn uptake[115].

2.2 Plant specific approaches

2.2.1. Selection and breeding of plants with low uptake potential

Since crop species and cultivars differ in their genetic tendency to take up TE, selection and breeding of crops for their low uptake potential open up new opportunities to minimize harmful elements in food chain. Large genetic differences occur among 200 sunflower genotypes for kernel Cd concentration[116]. The average Cd concentration of the five lowest genotypes is four fold lower than that of the five highest ones. Similarly 49 rice cultivars grow under simulated upland conditions on Cd contaminated soils differ in Cd concentration[117]. The differences in rice grain Cd among cultivars are much higher than in roots and stems[118]. Wheat variety trials across Australia showed genetic differences among cultivars in grain Cd [119.] Low Cd cultivars tended to have similar pedigrees, indicating the potential for selecting lines for low Cd concentration. Differences among plant cultivars in secretion of low molecular weight organic acids may influence root uptake of Cd[120]. Root Cd uptake may restrict translocation to stem, leaves, fruits and grains[121, 122]. Low Cd rice cultivars retain more Cd in roots and translocate less to grain than high Cd cultivars[123]. Differences in Cd concentration in durum wheat cultivars are attributed to differences in translocation from the root to the shoot and within the shoot, rather than to differences in root uptake[124]. High cation exchange capacity of roots can cause high grain Cd in wheat[21].

In spite of genetic variations in Cd uptake by cultivars, limited efforts were made to use selection or breeding to reduce Cd in crops in the past. Greater emphasis is now placed in finding low Cd cultivars of grain crops including wheat[20], durum wheat[125, 126 22], rice[122, 123], and soybean [123].. Cadmium uptake by maize in the mature stage had a significant genetic variation[127] other research is made on rapeseed[128] and lettuce [129.]

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The crossing program was developed near –isogenic high /low gain Cd concentration from five durum wheat crosses by Clarke et al.(1997)[130] . Each high/low pair was genetically uniform except the Cd concentration trait. The average grain Cd concentration was about 2.5 times greater for the high than for the low isolines (Table 3) [126]. The low –Cd uptake trait had

no effect on yield, protein content and kernel yield. The low –Cd uptake trait had no consistent effect on grain concentration of other TE but some indication that low –Cd trait may also be associated with low Zn accumulation under Zn deficient condition in solution culture experiment[131]. But in field, low Zn and Cu supply results in higher wheat grain Cd [132].

Even though cultivar selection can efficiently reduce Cd concentration in food crops (phytoexclusion), there are many constraints in utilizing this option. It is a time consuming process and low Cd traits of a cultivar must meet the requirement of acceptable yield level, agronomic suitability, and quality and disease resistance[22]. In wheat, grain Zn across cultivars ranges by a factor 1.6 which is lower than for grain Cd (factor 2-4 depending on year) [133] . Furthermore, both low- and high-Cd cultivars will be influenced by soil type, management practice, and yearly climatic conditions[87, 20.] Therefore combining management practices and use of low–Cd cultivars would be more effective in reducing Cd movement into the food chain[22].

2.2.2. Molecular and physiological aspect of TE transport

In plants, even at low concentrations, Cd accumulation can cause serious damages, like leaf chlorosis and necrosis, and can affect growth and development. Other Cd effects occur in plants such as breakdown of the photosynthesis apparatus, reduced respiration, indirect production of reactive oxygen species, DNA interaction, replacement of Zn and Fe as prosthetic groups and interaction with thiols. To limit Cd toxicity, plants have developed different strategies like exclusion, formation of complexes, compartmentalization and sequestration[134].

Different strategies for limiting potentially toxic TE uptake in the edible plant parts of can be envisaged at various levels.

i) restriction of TE movement to roots by mycorrhizas, binding metals to the cell wall and root exudates;

ii) ii) reduction of influx across the root plasma membrane or active efflux into the apoplast and finally to the soil,

iii) iii) Increased TE chelation in the cytosol by various ligands activated TE transport and accumulation in vacuoles to fix TE in non-edible plant parts, modulation of long-distance TE transport in order either to reduce the TE transport to edible parts or increase the flux back to non edible plant parts (Fig 2).

2.2.2.1 Cadmium uptake The role of IRT1

Epidermal cells constitute the main barrier between the soil and the plant. Transporters that are not specific enough to recognize only one of the required micronutrients but recognize and transport also non essential TE probably play a central role in plant survival as well as for human diets. The Cd

uptake is mediated by transporters or channels for other divalent cations[135]. Particularly several of the Zn and Fe transporting ZIP (ZRT, IRT-like Protein) gene products transport Cd with a wide range of affinities[136,137]. One of the first members identified in this family is IRT1 (iron-regulated transporter), an *Arabidopsis* cation transporter expressed in the roots of iron deficient plants[138]. AtIRT1 is essential for Fe acquisition from the soil in nongrass plants such as *A. thaliana* but also in rice, which as a strategy II plant takes up iron both as Fe^{2+} as well as Fe-phytosiderophore[139,140,141,142.] IRT1 is able to transport several divalent metal ions, including Cd, Co, Mn, and Zn[143]. Under Fe-deficient conditions, strategy I plants acidify the soil through the activation of a specific plasma membrane H^{+}-ATPase localized in root epidermal cells, potentially encoded by the *AHA2* gene in *Arabidopsis*[144] . Consequently, iron solubility increases, and Fe^{3+} is reduced by a specific reductase in order to be converted into the transportable Fe^{2+} form. Fe^{3+} reductase activity is probably the best studied among the different plasma membrane reductases[145]. FRO2 is the enzyme responsible for the plasma membrane Fe (III) reductase activity that is induced under Fe deficiency in the Arabidopsis roots [146]. In Arabidopsis, FRO2 is regulated both transcriptionally and post-transcriptionally.

Plants grown under iron-deficiency accumulate a variety of cations, including Cd[147]. This is directly linked to the incomplete IRT1 selectivity. Under iron deficiency the ratio of Fe^{2+} to Cd^{2+} is changed in favour of Cd^{2+} and consequently proportionally more Cd^{2+} is taken up. *Arabidopsis thaliana* overexpressing *AtIRT1* under the control of the 35S promoter accumulates larger amounts of Cd than wild-type plants rendering then hypersensitive, which evidences the role of IRT1 in Cd uptake[148]. Heterologous expression of IRT1 in *S. cerevisiae* previously indicates its contribution to the Cd^{2+}uptake[143]. *IRT1* and *FRO2* expression is repressed by Cd[126.]. In contrast to iron, Cd (II) cannot change the redox state, thus it is unlikely that FRO2 exhibits a function in the Cd uptake. A hypothesis is that FRO2 and IRT1 form a complex that is only stable when both proteins are present in the membrane [1149] Other ZIP family members may contribute to Cd uptake, although to a lower degree. Heterologous expression of AtZIP1, AtZIP2, AtZIP3 and TcZNT1 in *S. cerevisiae* shows that Zn^{2+} uptake activity is partially blocked by Cd^{2+}[150]. In yeast these transporters mediate high-affinity Zn^{2+} uptake and low-affinity Cd^{2+} uptake[151]. Respectively, suggesting the contribution of other ZIP transporters to Cd^{2+} uptake. Additionally, ZIP transporter, like AhZIP9 and AhZIP6 which are also present in the shoot, could be involved in Cd root-to-shoot transport and xylem unloading processes as well.

Iron is an essential micronutrient with a limited labile pool in many soils. Therefore, many studies have been carried out to understand the transport mechanisms and the regulation of IRT1 [152,153]. Detailed studies of the ZIP proteins expressing different mutated forms in yeast demonstrate that some of the residues are important for substrate recognition and transport activity[154.] For example the strain mutated expressing IRT1 in both, the D100A and E103A is less sensitive to Cd than either single

mutant and transports Zn but not Fe or Mn. Plaza et al. (2007)[155] expressed two AtIRT1 homologues from two different ecotypes of the hyperaccumulator *Thlaspi caerulescens* and showed that the two gene products conferred different Cd sensitivity to yeast. Detailed knowledge about IRT1 would allow developing or selecting plants that take up more specifically Fe, exclude non essential metals and have normal or increased contents of essential minerals in the edible parts. In contrast ferritin overexpression can enhance Cd uptake[156].

2.2.2.2. ABC transporters involved in heavy metal tolerance.

ABC transporters are one of the largest families of proteins in living organisms ranging from bacteria to humans[157]. ABC proteins are defined by the presence of an ATP binding cassette, also several highly conserved [159,159]. The majority of ABC genes encode membrane-bound proteins that participate in the transport of a wide range of molecules across membranes 160,161,162,163

Various types of ABC transporters are involved in TE resistance and particularly in Cd resistance processes. Two yeast ATP-binding cassette (ABC) transporters sequester metals into vacuoles. ScYCF1 is an ABC transporter of *S. cerevisiae* that contributes to Cd resistance by pumping glutathione-conjugated Cd into the vacuole[164.] In contrast, SpHMT1, a half-size ABC transporter of *Schizosaccharomyces pombe*, is a vacuolar phytochelatin–Cd complexe transporter[165]. Compared to wild-type plants, YCF1 overexpressing plants of *A. thaliana* exhibit increased Cd and Pb levels in shoots[166]. Additionally, vacuoles from transgenic plants exhibit a higher bis-glutathionate-Cd transport activity. The plant ABC transporters, AtMRP3[167] and AtATM3[168] have been suggested to transport Cd^{2+}. AtMRP3 partially restores Cd resistance when expressed in the ycf1 mutant [169], and the mitochondrial ABC transporter AtATM3 confers Cd and Pb resistance when overexpressed in *Arabidopsis*[168] and is also implicated in Fe homeostasis[170.] Gaillard et al. (2008) [171] suggest that *AtMRP6* is part of a cluster of ABC transporters involved in metal tolerance.

The pleiotropic drug resistance (PDR) subfamily is only found in yeast and plants[172]. All yeast and plant PDR proteins that have been localized so far reside in the plasma membrane and are regulated by various stimuli[173]. Several members of this genes family are also regulated by essential or non-essential metals. NtPDR3 is an iron-deficiency inducible ABC transporter in *Nicotiana tabacum*[174] .The expression of OsPDR9 is markedly induced by Zn and Cd in rice[175] and AtPDR12 contributes to Pb resistance in *A. thaliana*[176]. Based on membrane localization and mutant analysis, Pb resistance is related to AtPDR12. Overexpression of this gene reduces the Pb content, suggesting that AtPDR12 functions as a Pb (II) or Pb (II)-chelate extrusion at the root level. Particularly interesting is the fate of PDR8. This PDR is involved in pathogen resistance[177, 178,] and PDR8 is also implicated in Cd^{2+} and Pb^{2+} resistance[179]. AtPDR8-overexpressing plants are resistant to Cd^{2+} and have reduced shoot and root Cd contents compared to wild-type, knockout or silencing plants. The strong expression in the root epidermal cells is probably a main reason for the decreased amount of Cd in AtPDR8-over-

expressing plants. Consequently, AtPDR8 may confer Cd^{2+} resistance by extruding Cd^{2+} or Cd conjugates from the cytosol back to the soil. Moreover AtPDR8 decreases Cd content more in the shoots than in the roots, which may be due to the root-shoot barrier, which allows only a limited transfer of Cd^{2+} to the shoot. A similar observation was made when the heavy-metal pumping ATPase ZntA was expressed under the control of the 35S promoter, which also conferred increased Cd^{2+} tolerance and reduced Cd^{2+} contents in *Arabidopsis* plants[180] , That AtPDR8 extrudes Cd^{2+} across the plasma membrane appears quite unique, since so far, no similar observation has been reported for any organism. Mechanisms involving AtPDR8 may have a practical impact, since they allow either to search for plant varieties with constitutively high expression of the AtPDR8 homolog or to produce AtPDR8-overexpressing plants to reduce Cd contents in plants.

2.2.2.3 TE chelation.

Inside the cytosol, the free concentration of most metal ions is very low. Metal ions entering root cells bind to functional groups which act as metal chelators. These can either be low molecular organic compounds or macromolecules such as proteins. Detoxification in plants and other organism's usualy occurs through chelating compounds such as metallothioneins, phytochelatins, and amino acids such as histidine and organic acids such as citrate and malate, nicotianamine and its derivatives, the phytosiderophores [181,182,183,184]. Methallothionins (MTs) are the most common metal chelators in the cytoplasm of plant and animal cells. They are small cysteine-rich proteins that bind a variety of TE and play a major role in plant metal homeostasis[181]

Phytochelatins are by far the most important Cd chelator in plant cells and therefore plays an important role in Cd detoxification. Phytochelatin is a low molecular weight compound synthesized from the tripeptide gluthatione, and chelates TE by complexing them to the thiol group. Phytochelatine synthase is etopically expressed in plant cells and activated by binding Cd or other toxic metals or metaloids. In most plants phytochelatin synthase synthase is not upregulated after exposure to cadmium, however in some plants phytoccheltaine synthesis is induced by the activation of the enzyme as well as by increasing its expression level [185]. Following inactivation of phytochelatin synthase, plants suffer from severe Cd^{2+} hypersensitivity as this heavy metal is no more efficiently complexed within the cytosol. As an example, the mutant line of Arabidopsis *cad1-3* that is deficient in PC synthesis shows a severe loss of Cd tolerance[186] Phytochelatin also provides protection against arsenate, but it is uncertain whether it contributes to tolerance against other toxic metals/metalloids. The phytochelatin/Cd complexes formed are subsequently transported into the vacuole (see below).

2.2.2.4 Cadmium transport at the vacuolar membrane.

CAX

The vacuole is supposed to be a main site of Cd^{2+} accumulation, and tonoplast cation/H+ antiporters are considered as one of the systems for Cd translocation from the cytoplasm to the vacuole where it is thought to be sequestered[187]. Even if PC-Cd complexes (LMW) are transport into the vacuole by HMT1, a half-size ABC transporter from *S. pombe*[188 165], and phytochelatins are produced also by plants, no protein responsible for the same activity has been identified so far in plants.

CAX transporters have originally been identified as vacuolar Ca^{2+}/H^{+} antiporters. They contain 11 predicted α-helices and several conserved histidine residues[189]. In plants a vacuolar Cd^{2+}/H^{+} antiport activity has been demonstrated[190] and; at least one of the CAX transporters catalyzes the exchange of Cd^{2+} and other cations with protons at the vacuolar membrane[191.] Although, *Arabidopsis* cation exchanger genes catalyze the exchange of Ca^{2+} with protons, they do not appear to encode ion-specific transporters and modification of a single amino acid (His^{338}) by site directed mutagenesis increases Cd selectivity of the strong Ca transporter sCAX1 (N-terminal truncation of CAX1 resulting in constitutive CAX1 activity) [192]. Comparison of seven *CAX* genes in *N. tabacum* cv. KY14 indicates that all transport Cd^{2+}, Ca^{2+}, Zn^{2+} and Mn^{2+} to varying degrees, but that CAX4 and CAX2 exhibit high Cd^{2+} transport activity and selectivity in root tonoplast vesicles[193]. AtCAX2 or AtCAX4 overexpressing tobacco plants under the control of different promoters are more Cd tolerant and accumulate more Cd in roots compared to control plants. In contrast, shoot Cd does not differ in seedlings of transgenic and wild-type plants grown in hydroponic culture in the presence of 0.02 or 3 μM Cd^{2+}. The lower leaves of mature plants expressing *AtCAX2* or *AtCAX4*, under the control of two different root-selective promoters grown in the field (no Cd^{2+} amendment to the soil) accumulate less Cd than the respective controls[194.] Korenkov en al. (2007) [193] suggested that CAX antiporters are not negatively impacted by high Cd, and that supplementation of tonoplast with AtCAX compensates somewhat for reduced tonoplast proton pump and proton leak, and thereby results in sufficient vacuolar Cd sequestration to provide higher tolerance. CAX2 and CAX4 expression affects the root-to-shoot Cd distribution and the amount of Cd taken up has a great impact on this distribution. These results assume that CAX transporters contribute to vacuolar Cd sequestration and that this vacuolar mechanism in root cell might reduce Cd^{2+} translocation to the shoot. Since substrate specificity of these transporters can be easily altered, CAX genes may be a target to increase the vacuolar metal sink in the root for decreasing shoots Cd contents.

NRAMP

NRAMP (natural resistance associated macrophage protein) proteins are a ubiquitous family of metal transporters present in bacteria, fungi, plants and animals[195]. The *Arabidopsis* genome contains seven members of the NRAMP family. Three NRAMP proteins are implicated in Fe transport[196, 197]. Based on their abilities to complement a Fe uptake mutant in yeast, AtNRAMP1, AtNRAMP3 and AtNRAMP4 are characterized as iron transporters[198, 199]. These genes confer also Cd uptake activity when expressed in *S. cerevisiae*. NRAMP4 as well as NRAMP3 are localized in the vacuolar membrane[200]. In contrast, AtNRAMP1 has a plastid targeting sequence. Overexpression of AtNRAMP1 increases the tolerance of plants to excessive Fe concentrations suggesting a role in Fe distribution rather than plastidic iron uptake[166]. As IRT1, AtNRAMP3 transports Cd^{2+} and is

upregulated under Fe deficiency [154]. The AtNRAMP3 overexpression down regulates the primary Fe uptake system, IRT1 and FRO2[199]. This suggests that the overexpression of AtNRAMP3 increases Fe levels in the cytosol thereby downregulating Fe deficiency-induced genes such as FRO2 and IRT1. In *A. thaliana* it results also in Cd^{2+} hypersensitivity. AtNRAMP3 and AtNRAMP4 exhibit redundant functions because the single mutants do not have obvious growth defects. In contrast, the double mutant is sensitive to Fe depletion and the phenotype is correlated with the level of Fe depletion. Double mutants are no more able to mobilize Fe stores from the vacuole early in development. This is a main constraint during germination, since the young plantlet depends on internal Fe stores. Results support that Cd can be remobilized from the vacuole into the cytosol via NRAMP transporters. Plants which translocate Fe during seed germination will also necessarily remobilize stored Cd. A sufficient Fe supply during seed production may lower the Cd translocation during the vegetative period. Alternatively, a better understanding of the substrate recognition by NRAMPs may allow engineering NRAMPs which will be more specific for Fe^{2+}, decreasing the translocation of vacuolar Cd^{2+}.

2.2.2.5Translocation of cadmium from the root to the shoot

The activity of metal-sequestering pathways in root cells likely play a key role in determining the rate of translocation to the aerial parts of the plant.

HMA4

Long-distance transport of inorganic nutrients plays a crucial role in plant development and metal distribution. This is the only way to deliver metals taken up by the root to the shoot. In the shoot, part of the nutrients and metals may be remobilized during further growth then during senescence for seeds production. The understanding of this process which is mediated by different transporters can be useful to select or engineer plants which have the capacity to accumulate or reduce non-essential metals in different tissues.

The Heavy-Metal transporting P1b-ATPase (HMAs) proteins are membrane proteins that use ATP to drive metal transport across biological membranes against their electrochemical gradient[201, 202.] In *Arabidopsis* three members of the HMA family, AtHMA2, 3 and 4, are classified as Zn^{2+}-ATPase. AtHMA4 was the first member of this group to be cloned and characterized[203]. It confers Cd^{2+} resistance in yeast and rescues the Zn deficiency of the *E. coli* zntA mutant suggesting a role in Zn^{2+} and Cd^{2+} transport. AtHMA3 confers Cd^{2+} and Pb^{2+} tolerance to Δycf1 yeast cells, being apparently located in the yeast vacuole membrane when fused with green fluorescence protein[204]. Thus, AtHMA3 likely participates in the vacuolar storage of Cd in plants. Interestingly, plants overexpressing *AtHMA3* improves tolerance to Cd, Co, Pb, Zn and Cd accumulation increases by about two to three-fold in plants overexpressing *AtHMA3* compared to wild-type plants[205]. While the hma2, hma3, hma4 single mutants do not show any obvious growth defects when grown in soil, hma2 hma4 double mutants exhibit a drastic phenotype. These plants are chlorotic and fail to set seeds[206]. *Hma2* mutants

accumulate exclusively more Zn and Cd than wild-type plants, *hma4* mutant plants accumulate more Zn and Cd in the roots but they accumulate less Zn and Cd in leaves[207.] This impaired distribution is more pronounced in *hma2 hma4* double mutants. Addition of excess Zn to the growth medium suppresses the growth defect, despite the fact that these double mutants still have consistently lower levels of Zn in the aerial portions of the plant. Additionally, plants overexpressing HMA4 have an increased tolerance to both Zn^{2+} and Cd^{2+}. HMA4 and HMA2 would mediate the efflux of Zn across the plasma membrane resulting in the xylem loading. Promoter GUS fusions show that these genes are expressed in the vascular bundles of roots and shoots. HMA2 and HMA4 reside in the plasma membrane[206, 207] and apart of their main function as Zn translocators to the shoot they may also transport Cd^{2+} to the shoot. This hypothesis is confirmed in the Zn/Cd hyperaccumulator *A. halleri*. In this plant, both metal hyperaccumulation depends on the metal pump HMA4 which is highly expressed due to a triplication of HMA4 and altered cis-regulatory elements. As for NRAMPs, plants require HMA4 homologues, since Zn has to be transferred to the shoot. However, it may be possible to screen for plants where the Zn/Cd ratio is altered and find elements in the HMA4 protein which affect the specificity for this transporter. Further studies on HMA4 will also reveal amino acids important for this specificity and allow engineering plants which have a reduced Cd translocation rate.

2.2.3 Plant roots and rhizospheric effects

2.2.3.1. Microbial transformation in soil and plants

Plants suffer nutrient deficiency stress when the essential TE availability in soil (and/or the amount of nutrients taken up) is lower than required for sustaining metabolic processes in a particular growth stage .Deficiency may occur as a result of (i) an inherently low TE amount in the soil, (ii) low TE mobility in the soil, or (iii) poor solubility of given chemical forms of the nutrients. Plants exposed to nutrient deficiency activate a range of mechanisms that result in increased nutrient availability in the rhizosphere compared with the bulk soil. Plants may change their root morphology, increase the affinity of nutrient transporters in the plasma membrane, and exude organic compounds (carboxylates, phenolics, carbohydrates, enzymes) and protons. Rhizosphere microorganisms influence TE availability where adding beneficial microorganisms may result in enhanced uptake of nutrients to crops. The redox potential of the rhizosphere dynamically changed due to the continuous release of oxygen by roots, especially under reduction conditions, such as in rice fields, and thus affects the chemical form of metals.

The microbial reduction of elements has attracted recent interest because these transformations can play crucial roles in the cycling of both inorganic and organic species in a

range of environments. If harnessed, it may offer the basis for a wide range of innovative biotechnological processes. Under certain conditions, however, microbial metal reduction can also mobilise non-essential metals with potentially harmful effects on human health[209].

Reduction and oxidation of Mn by microorganisms are important components of Mn cycling in soil. Fluorescent Pseudomonas is effective Mn reducers, which appear to be more abundant in the rhizospheres of some Mn-efficient *Triticum aestivum* genotypes compared with Mn-inefficient genotypes[210]. The composition of rhizosphere bacterial communities was assessed using the ribosomal intergenetic spacer analysis (RISA) region of the bacterial DNA 208. The results suggest the importance of microorganisms in plant Mn uptake.

The mobilization of arsenic from sediments to drinking water and to plants constitutes a major toxic hazard to millions of people in Bangladesh and West Bengal. Islam et al. (2004)[211] detected the role of indigenous metal-reducing bacteria in the formation of toxic, mobile As(III), in sediments through the use of a microcosm-based study. The addition of acetate to anaerobic sediments, as a proxy for OM and a potential electron donor for metal reduction, resulted in stimulation of the microbial reduction of Fe (III), followed by As (V) reduction and release of As (III). These results suggest that either direct enzymatic microbial reduction of As (V) by Fe (III)-reducing bacteria or indirect mechanisms associated with the reduction of Fe (III) oxides could be important mechanisms for arsenic release in these sediments, with the involvement of Geobacter species implicated in these transformations. Although Geobacter species have not been reported to reduce As(V), these organisms do have the physiological capacity to reduce a wide range of metals and metalloids[212,209], vis-a-vis a battery of c-type cytochromes[213], while the existence of an As resistance operon, including a gene for a putative arsenate reductase (arsC), was reported for *G. sulfurreducens*[213]. The potential of G. sulfurreducens to mobilize as vis-a-vis direct enzymatic reduction and indirect mechanisms linked to Fe (III) reduction have been studied214

Although the full environmental relevance of TE transformation processes has only recently become apparent, rapid advances in the understanding of these important biotransformations have been made. However, we still have much to learn about the precise mechanisms involved and the full impact of such reactions on a range of biogeochemical cycles. Given the availability of genomic sequences for key metal-reducing micro-organisms, new post-genomic and proteomic approaches and the possibility of combining these tools with advanced techniques from other branches of science and technology are required.

2.2.3.2. Plant root interactions and rhizosphere effects

The study of TE uptake by plants, however, requires knowledge of the processes through which metals and metalloids are transferred to plant roots, including the rhizospheric processes, especially to base manipulation 215,216. Microorganisms may affect the TE bioavailability through their influence on (i) the growth and morphology of roots; (ii) the physiology and development of plants; (iii) the TE fractionation; and (iv) the root uptake process. Understanding the role of plant–microbe–soil interactions in governing nutrient availability in the rhizosphere will enhance the economic and environmental sustainability of crop production.

Root exudates selectively influence the growth of microorganisms that colonize the rhizosphere by altering the soil chemistry in the root vicinity and by serving as selective growth substrates for soil microorganisms. Microorganisms in turn influence the composition and quantity of various root exudate components through their effects on root cell leakage, cell metabolism, and plant nutrition. Based on differences in root exudation and rhizodeposition in different root zones, rhizosphere microbial communities can vary in structure and species composition in various root locations or in relation to soil type, plant species, nutritional status, age, stress, disease, and other environmental factors[217,218,219].

Cadmium accumulation varies between cultivars of durum wheat (*Triticum turgidum* var. durum), and low-molecular-weight organic acids (LMWOAs) produced at the soil-root interface may control the availability and uptake of Cd by these plants[220]. No water extractable LMWOAs are identified in the bulk soil indicating the importance of microbial-plant interaction in TE accumulation. Total amount of LMWOAs in the rhizosphere soil of the high Cd accumulator wheat cultivars is higher than that for the low Cd accumulator in all three soils, resulting in increased Cd uptake by the accumulator cultivar.

Rhizobacteria can play an essential role in the resistance of plants to stress induced by some trace elements. The inoculation of rape and brown mustard plants with rhizobacteria enhances the resistance of the plants to Ni, Pb, Zn, and Cd [221]. Seed bacterization with TE–resistant rhizobacteria strains such as *Azospirillum lipoferum*, *Arthrobacter mysorens*, *Agrobacterium radiobacter*, and *Flavobacterium* sp. improve the growth of barley plants and the nutrient uptake from Pb- and Cd-contaminated soil in lab and field conditions[222]. The bacterization also prevents the accumulation of Pb and Cd in barley plants, thereby mitigating the toxic effect of both metals on the plants.

The root exudation of organic compounds contributes to increase nutrient availability in the rhizosphere. The regulation of the complete exudation process and the underlying genetics need to be more elucidated. Fully understanding the interactions among root exudation, indigenous rhizosphere microorganisms, and TE availability is very crucial for crop production.

2.2.3.3. Microbial plant growth-promotion in contaminated soils

The application of bioinoculants like arbuscular-mycorrhizal fungus, and/or plant growth-promoting rhizobacteria such as *Azospirillum*, *Agrobacteria*, *Pseudomonas* and several *Bacillus* species is an environment-friendly, energy efficient and economically viable option for reclaiming soils and increasing biomass production 224,223. The inoculation of bacterial strains producing exo-polysaccharides enables plants to withstand the initial effects of excessive TE exposure and the osmotic stresses but it also benefits the inoculated plants in terms of an increased exploitation of the soil nutrients. By providing an increased extent of rhizodeposits in the soil, bioinoculants assist in initiating soil microbial activities[225].

Plant growth-promoting rhizobacteria (PGPR) associated with plant roots exert beneficial effects on plant growth and nutrition through a number of mechanisms such as N_2 fixation, production of phytohormones and siderophores, and transformation of nutrient elements when they are either applied to seeds or incorporated into the soil[226,227] . Also, some rhizobacteria can exude compounds, such as antibiotics, phosphate solubilization, indoleacetic acid (IAA), siderophores, 1-aminocyclopropane-1-carboxylic acid (ACC) deaminase , which increase bioavailability and facilitate root absorption of nutrients, such as Fe or non-essential elements, such as Cd and Pb[228] , and enhancing the tolerance of host plants by promoting plant growth[229,230] . IAA produced by rhizobacteria is believed to play an important role as a phytohormone, influencing many cellular plant processes. The biosynthesis of auxins with their excretion into soil mainly contributes to the bacterial plant-growth-promoting effect[231]. Fluorescent Pseudomonads produce siderophores, the pyoverdines which are available in both homologous and heterologous uptake systems [232]. These Pseudomonads are low molecular weight iron chelators which are released under iron limited conditions in their surroundings, possess high binding affinity and specificity for iron (III), and facilitate its transport into the bacterial cell [223]. All these bacterial characteristics support the symbiotic interactions in the rhizosphere zone for mutual benefits of plants and microbes.

Some plant growth-promoting bacteria, i.e. free-living soil bacteria that are involved in a beneficial association with plants, contain the enzyme ACC deaminase[234], which can cleave the plant ethylene precursor ACC and lower the level of ethylene in a developing or stressed plant by excessive TE exposure. PGPR containing ACC deaminase may insure that the ethylene level does not impair root growth[235], and by facilitating the formation of longer roots may enhance seedling survival and plant root growth. PGPR stimulate root growth of different crop plants including sunflower and maize [221]. The bacteria utilize the ammonia evolved from ACC as a nitrogen source and thereby decrease ACC within the plant[236.] PGPR dispose different mechanisms to suppress the development of plant-root pathogens[237].

3.0 Regulatory control of trace element entry to food chain

Most of the European soil protection and soil contamination legislations were promulgated in "nineteen nineties". In case of suspected contamination, soil investigation mostly follows stepwise approach starting with a preliminary investigation, then in-depth investigation and at the end final remediation. The main purposes of regulatory values of TE are to reduce their accumulation rate in European agricultural soils on one hand and to grow consumable crops that do not pose adverse and unacceptable risk to animal and human health on the other. Such control values are, however, complicated by (i) soil organisms differ in their sensitivity to metals, (ii) exposure pathways differ for TE, and (iii) properties of soil and materials contaminated with TE (e.g. fertilizer, biosolids etc.) influence the degree of exposure (bioavailability) of metals and metalloids in soil and different chemically nature of trace elements. The precautionary option adopted by Scandinavian countries is the maintenance of the status quo in terms of metal concentration in soil, implying that input must not exceed output of metals. This other side of sustainability is not easily achievable. Furthermore, the feasibility of such option can be restricted in many countries because of the presence of contamination sources, which are yet to be minimized and for economic reasons. The alternative EU option is to regulate TE concentration in soils to levels that will maintain environmental health for agricultural purposes and also avoid any off-site impacts due to movement of contaminants to interlinked ecosystems such as water, air etc. It is strongly based on observed TE impacts. However, the major consideration for regulatory authorities is that contamination of agricultural soils by TE is irreversible.

The soil standards in most of the countries should trigger gentle and hard actions.

Differences in selected software model, (standard) parameters values, selected human toxicological and ecotoxicological criteria are reason for a substantial variation in soil standard generic values and clean-up standards for TE from country to country [3]. Toxicity thresholds based on the free metal ion activity vary more than those expressed on total soil metal; these ones are explained but not predicted using the concept of the biotic ligand model and rise in line with the cation exchange capacity and contaminant aging in the soil[17]. In Switzerland and Germany, there are two types of generic values, i.e. one based on total and the other based on neutral salt extractable values (derived from adverse effects on plant and soil organisms). The soil generic values should be in conjunction with the crop values and vice versa. This will help in achieving model input =output.

Most of the generic soil standard values differ widely and do not account for the interfacial interaction of contaminants. Sometimes these values are selected on political basis rather than scientific background. Furthermore, these values should be used as a basis for prevention rather than complete cure. The generic values should safe guard the growth of very sensitive organisms. Efforts should be made to harmonize selection and basis of development of generic values. In this direction the efforts made by the International Standardization Organization (ISO) and EU Joint Research Centre are appreciable [238,239]. Many single background concentrations have been defined for a country, which could give rise to either overestimation or underestimation of metal contamination and the associated risk for a particular soil. Regional guidelines for TE in soils, accounting for soil types and subjacent rock stone, are proposed to better assess soil contamination [240,241,242,243].

For a sustainable soil quality, able to grow healthy plants, serious efforts to improve the quality of agricultural inputs as well as on air quality, water quality and soil organisms are needed.

The TWI (Total Weekly Intake) of 7 µg Cd per kilogram of body weight (µg/kg BW) set by the Joint FAO/WHO Expert Committee on Food Additives (JECFA) in 1988 and reaffirmed in 1995 is used by the EU. In January 2009, the EFSA's panel on contaminants in the food chain has however reduced it to 2.5 µg/kg BW [244]. Risk assessors would consider this new value, so this could affect decisions for ingredients grown in contaminated areas.

Grains, vegetables, pulses and nuts, as well as meat are frequently consumed foods which may come with a high Cd content. Other foods, e.g. fish, chocolate, mushroom and dietary supplements, may have a high content but are less often eaten. The new TWI of 2.5 µg/kg bw set by the panel is based on studies investigating levels of Cd in the urine and levels

of beta-2-microglobulin, a protein that indicates kidney function, and data translation to actual dietary exposure[244]. This TWI considers early indicators of a change in kidney function and not its damage. Therefore, even though exposure should be reduced, the risk of actual kidney damage from exceeding the TWI is very low. Data on Cd in food in 20 countries and consumption data were reviewed. High Cd exposure reaches 3.0 μg/kg per week and in average 2.3 μg/kg bw, but vegetarians could eat as much as 5.4 μg/kg bw. Children tend to eat more food per kg bw and could exceed the TWI.

The Food Standards Agency's Committee on Toxicity of Chemicals in Food, Consumer Products and the Environment (COT) surveyed in 2006 24 elements including metals in samples of 20 different food groups, bought in 24 randomly selected UK towns for estimating the dietary exposures of the elements for UK consumers [245]. There were no specific health concerns associated with the findings, which showed that levels of most of the elements in UK food groups were the same or lower than in previous total diet study conducted in 2000. The COT recommended that (1) future research should take in information on aluminium and its different forms, barium (e.g. in nuts) and manganese (e.g. in beverages) in food and how bioavailable they are; (2) large variability in Al in food should be "clarified," and attention should be paid to whether this represents an increasing trend; and (3) efforts should continue to reduce dietary exposure to inorganic As and to Pb.

4.0 Summary and conclusions

Trace elements and especially Cd in human food-chain are of major concern and thus restricting their entry into food chain and protecting human and animal health is an important challenge for agronomists, microbiologists, plant biologists and physiochemists. The in situ stabilization of TE in soils by amendments such as lime, OM, phosphates, and mineral oxides can reduce the bioavailable TE fractions in soils and their entry into food crops; however, the effects depend on soil conditions, plant species and the management practices. Similarly, element interactions affect TE in food crops. Zinc application reduces Cd uptake by 40% and 60% in flax and durum wheat, respectively, and Cd translocation to the seed/grain in both crops (> 30%). Grain Cd increases when wheat is grown after lupins and thus a proper crop rotation is essential to minimize Cd uptake. Although the decontamination techniques such as phytoremediation by the use of hyperaccumulators or high biomass crops and solubilization by ligands (e.g. ion exchange resins, natural and synthetic chelators) provide alternative options for reducing TE transfer to food crops, they suffer with several limitations.

Phytoremediation is a slow and long-term process to achieve the remediation objectives, whereas solubilization may have undesired side-effects such as increasing the TE toxicity and their leaching to deeper soil layers or to groundwater.

Selection and breeding of crops for their low TE uptake potential (phytoexclusion) can minimize non-essential elements in food chain. Large genetic variations exist for Cd uptake among cultivars, e.g. low Cd rice cultivars retain more Cd in roots and translocate less to grain than high Cd cultivars. Phytoexclusion has many constraints, one being the time consuming process of plant selection because excluder cultivars must also meet the requirement of suitable yield level, agronomic suitability, quality and disease resistance, etc.

Knowledge of the identity of transporters for various TE is increasing through the use of molecular genetic techniques. However TE-ligand speciation and TE transport in plants are dynamic processes varying across tissues, subcellular compartments, developmental stages, and plant species. The increased availability of gene deletion mutants, or plants over- or underexpressing certain key genes or chimeric genes under the control of different promoters will provide evidence in relation to Cd translocation and accumulation. Finding the responsible genes for low Cd content in edible plant parts is a target of future plant breeding programs. The root exudates contribute to nutrient availability in the rhizosphere. The rhizodeposition regulation and the underlying genetics need more insights. Fully understanding of the interactions between root exudation, indigenous rhizosphere microorganisms, and TE availability is crucial for crop production. Bioengineering the rhizosphere by adding beneficial microorganisms will require understanding of microbe–microbe and microbe–plant interactions, enabling introduced microorganisms to show full activity in the targeted rhizosphere to improve crop production and yields. In spite of complex web of geochemical and biological interactions, which determine the TE bioavailability in soil-plant-human system, we conclude that soil and plant specific options must act in synergy to reduce TE transfer to the food chain.

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Table1. *Different categories of remediation techniques for soils polluted by trace elements (Gupta et a. 1999 [238])*

1. In-Situ Gentle		2. In-Situ Harsh	3. In-Situ Harsh
1.1. **Stabilization**	***1.2.*** **Decontamination**	**Soil Use Restrictive Measures** (*in situ*)	**Soil Destructive Measures** (*ex- or in situ*)
1.1.1. **Increase of soil pH by liming** ***1.1.2.*** **Increase of binding capacity** by Clays, Oxides, Zeolites, clean waste containing clay and oxides (e.g. gravel sludge) ***1.1.3.*** **Plants** ***1.1.4*** **Microorganisms**	***1.2.1.*** **Controlled and targeted mobilization** with natural and synthetic acidifying, and complexing agents ***1.2.2.*** **Capture of mobilised metals** with plants or natural and synthetic captors ***1.2.3.*** **Harvesting of the metal-loaded captors**	***2.1.*** **Land use ban and limitation** ***2.2.*** **Land use change** ***2.3.*** **Seal and close**	***3.1.*** **Incineration** ***3.2.*** **Deposition** of contaminated layers striped off ***3.3.*** **Thermal treatment** ***3.4.*** **Electromigration** ***3.5.*** **Soil washing** ***3.6.*** **Vitrification** ***3.7.*** **Deep Ploughing** ***3.8.*** **Dilution**

Table 2. Trace element concentration (mg kg^{-1}) wheat grain after liming to different pH levels in a naturally metal rich moraine soil (Extracted from Singh et al.(1995)[28]).

Soil pH	Cd	Ni.	Zn	Cu
5.5	1.34	1.39	47.8	4.6
6.5	0.55	0.83	41.9	3.2
7.0	0.55	0.76	31.6	4.0
7.5	0.52	0.84	27.3	4.3
$LSD_{0.05}$	0.31	0.36	12.4	2.2

Table 3. Grain Cd concentration in durum isogenic pairs and parent grown in varying environments (Modified from Grant et al. 2008)

Genotype[#]	Casselton	Regina	Swift current	Stewart valley
8982-SF-L	0.09	0.11	0.95	.0.05
8982-SF-H	0.21	0.33	0.11	0.15
8982-TL-L	0.10	0.11	0.04	0.08
8982-TL-H	0.28	0.34	0.11	0.18
W9260-BC-L	0.14	0.19	0.10	0.03
W9260-BC-H	0.30	0.32	0.14	0.07
W9261-BG-L	0.10	0.14	0.05	0.13
W9261-BG-H	0.27	0.27	0.10	0.23
W9262-339A-L	0.08	0.10	0.03	0.20
W9262-339A-H	0.23	0.30	0.11	0.07
Contrast H vs L[§]	**	**	**	**

Isogenic lines designations ending “L” indicate low-Cd accumulator and “H” indicates high accumulator.

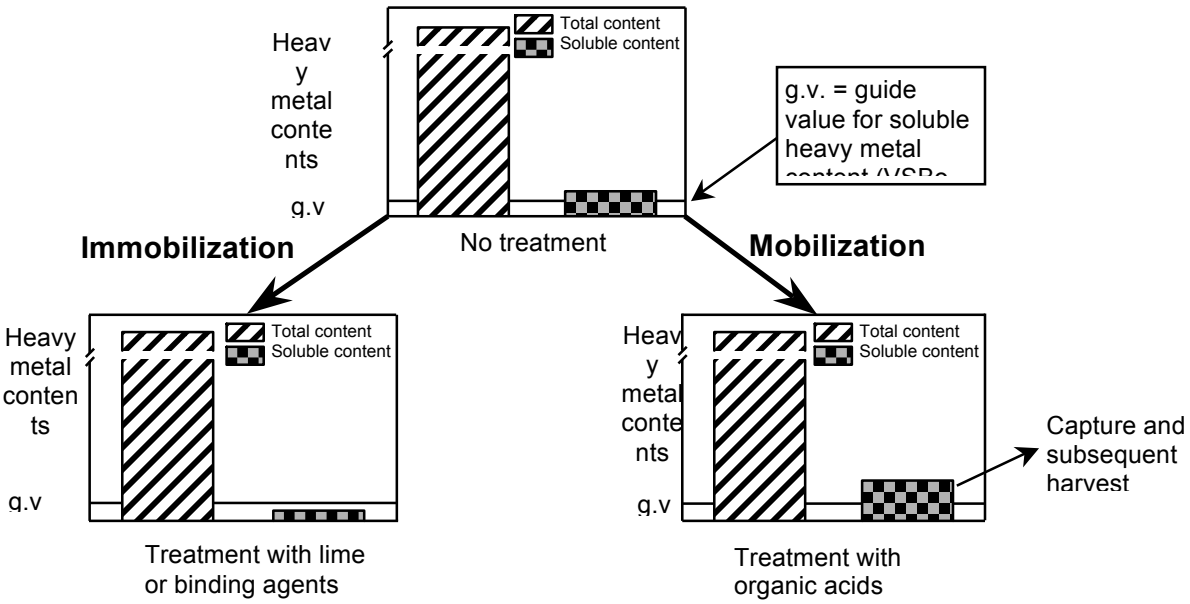
§ Significance of high vs. low isolines ($P<0.01$)

Figure captions

Figure 1. Effect of remediation treatments on mobilization/immobilization of metals (Adopted from Alloway, 1995 [239]).

Figure2. Schematic representation of Cd uptake and translocation in plant roots. Cd^{2+} is taken up into the plant by ZIP transporters (IRT1, ZIP1-4, ZNT1, 2 are good candidates) and possibly by Ca^{2+} channels. In the cytosol, the main part of Cd is chelated with GSH to form bisglutathionato-Cd complexes (GS_2-Cd) and other unknown molecules (?-Cd). GS_2-Cd can interact with PC synthase resulting in the formation of PC-Cd (LMW) complexes. Chelation and/or sequestration processes by ferritins, metallothioneins and small molecules such citrate are postulated in plants, but they are not mentioned here. Also, presence of free Cd ions is supposed to be very limited due to the physiological conditions in the cytosol. Furthermore, Cd^{2+} may also interact with Ca^{2+} binding proteins.
For detoxification, Cd^{2+} or Cd-conjugates could be remobilized from the cytoplasm into the apoplast by ABC transporters (PDR8) or sequestered into the vacuole. For the latter, two different pathways are postulated, GS_2-Cd and/or LMW complexes are hypothesized to be transported into the vacuole by ABC transporters not characterized yet, or Cd^{2+} could be sequestered into the vacuole by Cd^{2+}/H^+ antiporters (AtCAX2 and AtCAX4 exhibit the highest Cd (II) transport activity). At least a part of vacuolar Cd is bound in HMW and in yet unidentified complexes (?-Cd). However, a fraction of vacuolar Cd can be remobilized into the cytosol by Nramp transporters which are upregulated under Fe starvation (Nramp1, 3, 4).
The efficiency of the sequestration and exclusion processes determines the amount of Cd that will be transferred to the aerial parts of the plant. Loading of the xylem with Cd occurs by HMA-type plasma membrane efflux pumps (HMA2, 4) and an efflux of PC-Cd or GS_2-Cd complexes from the cytosol to the xylem sap is possibly mediated by an unknown transporter. In the xylem Cd is bound to so far unknown ligands (?-Cd).
Similar mechanisms are occurring in aerial parts of the plant where some of the transporters described above or their homologues have similar functions. Phloem-loading and unloading of Cd(II) is postulated but is only poorly described.

Figure 1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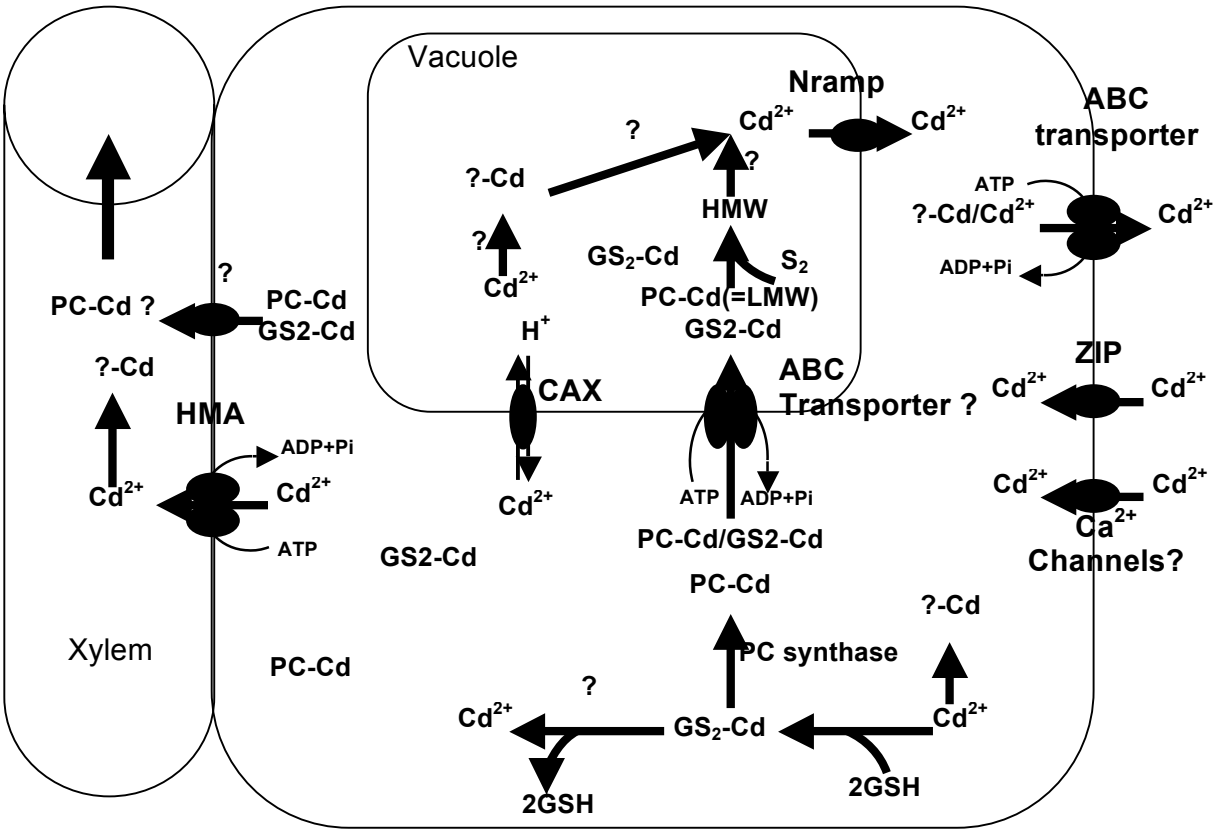
harvest
Treatment with lime
or binding agents
Treatment with
organic acids

Figure 2


Vacuole
Nramp
ABC transporter
?
Cd2+
Cd2+
?-Cd
?
HMW
ATP
?-Cd/Cd2+
Cd2+
ADP+Pi
?
Cd2+
GS2-Cd
S2
PC-Cd(=LMW)
GS2-Cd
PC-Cd ?
?
PC-Cd
GS2-Cd
H+
CAX
ABC Transporter ?
ZIP
?-Cd
HMA
Cd2+
Cd2+
ADP+Pi
Cd2+
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ATP
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ATP
ADP+Pi
Ca2+ Channels?
GS2-Cd
PC-Cd/GS2-Cd
PC-Cd
?-Cd
Xylem
PC-Cd
PC synthase
?
Cd2+
GS2-Cd
Cd2+
2GSH
2GSH